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Proliferative characteristics of the ependymal layer during the early development of the spinal cord in the mouse

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INTRODUCTION

The efficiency of the nervous system depends partly on the quality of its cells and partly on their quantity, and in terms of function these properties are closely interrelated. However, the quantitative aspect can readily be examined separately from the much more subtle process of qualitative variation. The investigations reported here were prompted by a consideration of processes known to be involved in vertebrate neurogenesis, which seemed to the author to impose effective limitations on cell production, and which have been discussed in brief notes elsewhere (Smart, 1965, 1971*a*). The approach consists in asking the question 'What limitations are encountered by the cell-producing mechanism of the neural tube if it is required to produce the maximum number of nerve cells, and how can the system be modified to improve cell production?' Although the proposition was not put to the central nervous system by the evolutionary process in quite such a circumscribed way, it is an approach which seems to produce some interesting results, and can be regarded as a useful heuristic device, if no more.

The argument proceeds from two major facts of neurogenesis: (1) that nerve cells do not themselves divide but stem from a population of undifferentiated precursor cells, forming a spatially separate proliferative compartment, which exists only for a short space of time at the beginning of the animal's life span, and (2) that this compartment, namely the ependymal layer, is a pseudostratified epithelium and its proliferative processes are governed by the morphological constraints of its epithelial context. These statements require some amplification and this is now given.

(1) *Proliferative characteristics.* Two basic possibilities are open to an undifferentiated ependymal cell; it can differentiate and lose the power of further division, or it can divide to give two daughter cells, each of which is again faced with the possibility of differentiation or division. This gives rise to two basic types of cell population; one which is given over entirely to proliferation, and one which contains a greater or lesser element of differentiation. In Figs. 1(*a*) and (*b*) two such populations are compared. A population which is dividing without differentiation as in Fig. 1(*a*) can obviously produce more cells in a given number of generations than one which is losing cells from the proliferative compartment by differentiation as in Fig. 1(*b*). As neuron production is limited to a short period at the beginning of the animal's life span, only a limited number of generations of cells is available in which to furnish the animal with its full complement of nerve cells, and therefore on numerical grounds,

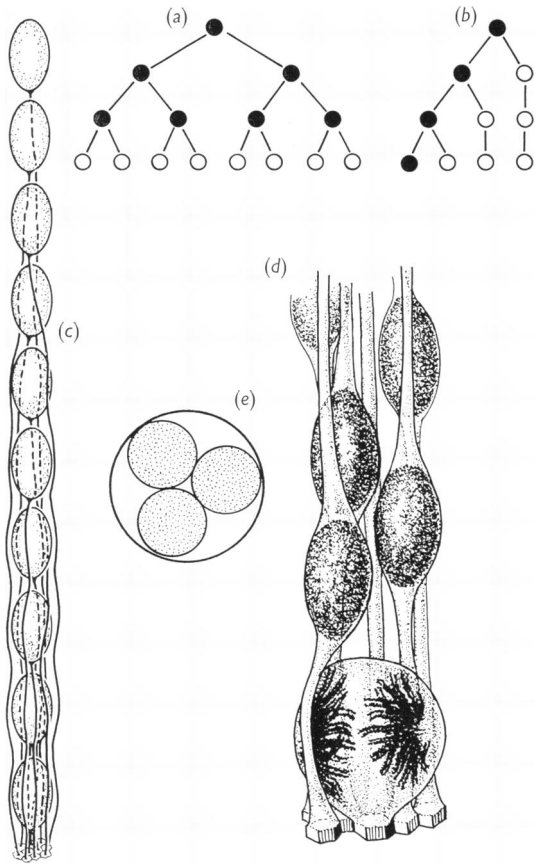


Fig. 1. (*a*) Diagram depicting a population of undifferentiated ependymal cells (represented by solid black roundels) dividing to give undifferentiated daughter cells, which continue to divide until the last generation, when they differentiate into nerve cells (represented as open circles), which thereafter lose the power of further division. The precursor population grows and then disappears abruptly. After three generations the model in the diagram has produced eight neurons.

(*b*) Diagram representing a population of ependymal cells in which proliferation and differentiation are balanced. At each generation one daughter cell differentiates into a neuron and loses the power of division while the other remains undifferentiated and continues to divide. The precursor population remains steady and the neuron population grows. After three generations this system consists of one undifferentiated cell and three neurons.

(*c*) Diagrammatic representation of an area of the ependymal layer pseudostratified to a depth of ten nuclei. The apical processes of the cells occupy an area of the central canal surface about the same as the cross-section area of an ependymal nucleus.

(*d*) Three-dimensional diagram of ependymal cells with hexagonal terminal bar network at cell apices. One cell is in mitosis (anaphase) and its volume has increased.

(*e*) Diagram to show size relation of mitotic and interphase ependymal cells. The outer circle and three inscribed stippled circles correspond in cross-sectional area to the relative sizes of mitotic and interphase cells respectively. The diagram is essentially a plan view of (*d*).

it would seem advantageous to the nervous system to increase its investment in undifferentiated cells for as long as possible. *The first rule of neurogenesis in the central nervous system would, therefore, appear to be that, in order to produce the maximum number of nerve cells in a given number of generations, differentiation must be delayed.*

(2) *The epithelial context.* From the foregoing it is apparent that the delayed differentiation required for increased cell production will be associated with a proliferative compartment of increasing size as in Fig. 1(*c*). Structurally this proliferative compartment is a pseudostratified epithelium; that is, although there may be several ayers of nuclei in the ependymal layer of the neural tube, the cytoplasm belonging to each nucleus makes contact with both the apical and basal surfaces of the epithelium. When a cell in the ependymal layer goes into mitosis, the nucleus migrates to the apical pole of the cell and the cytoplasm withdraws its peripheral process, rounding-up towards the firm attachments of the terminal bars at the cell apex (Fig. 1*d*). Here the cytoplasm divides, a new segment of terminal bar is fashioned, and the two daughter nuclei migrate back to the periphery of the layer (Sauer, 1935, 1936; Sauer & Walker, 1959; Hinds & Ruffett, 1971). This process results in all mitotic figures in the ependymal layer striving to locate themselves at the surface of the central canal (Fig. 1*d*). In regions of the neural tube where the area of the surface of the central canal does not increase as rapidly as cell proliferation increases the volume of the ependymal layer, an increase in depth of the layer will occur, and, because of the foregoing characteristics, this increasing pseudostratification will create the following disproportions between cell number and the canal surface.

(*a*) As progressive pseudostratification is a process in which an increasing number of cells is related to a constant area of central canal, a point will eventually be reached when each unit area of canal surface is accommodating the maximum number of cell apices it can, i.e. the mesh of the hexagonal terminal bar network will get finer and finer until it is no longer capable of further subdivision for the admission of further cell apices. For example, in the alar lamina of the early mouse neural tube, where pseudostratification reaches a depth of ten nuclei, the apical processes of ten cells share an area of canal surface of about the same area as the cross-sectional area of one nucleus (Fig. 1*c*). The short diameter of an ellipsoidal ependymal nucleus was found by measurement to be about 5 μm and its cross-sectional area is therefore about 20 μm^2. Thus the average allocation of canal surface per cell apex is about 2·0 μm^2 and the average breadth of an apex is about 0·8 μm. As the depth of a terminal bar complex is about 0·5 μm, or half the diameter of the apex, further subdivision would mean that the depth of the terminal bar would approach and then exceed the diameter of the diminishing apex. It would seem probable that after attaining a depth of ten nuclei, pseudostratification runs into problems of disproportion of scale at the cell apices.

(*b*) If, as the ependymal cell population grows, the mitotic index, i.e. the number of mitotic figures per 100 nuclei, remains the same, the number of mitotic figures in the population will increase until all the available surface of the central canal is occupied by them. From observation (e.g. Fig. 12), certain areas of the surface of the central canal appear to be saturated with mitotic figures. If, in these areas, mitosis continues to be restricted to the apical location, this will result in a lowering of the mitotic index as there will be no room to accommodate the additional mitotic figures generated by the increasing cell population.

(*c*) A cell population may increase its proliferative capacity by reducing the duration of mitosis and interphase. If this should occur in a densely packed area of the ependymal layer, the following disproportion between the migratory and mitotic

cycles becomes evident: it is best explained by reference to the three-dimensional diagram in Fig. 1(*d*). It was found by measurement that the diameter of a globular mitotic cell at the central canal surface was about 10 μm. Thus, in an area of the neural epithelium where pseudostratification is five nuclei deep and packing is dense, each mitotic figure lies beneath a column of 15 interphase nuclei (Figs. 1*d*, *e*). Since all cells seem to be participating in the proliferative process, as indicated by their uptake of ^{3}H-thymidine (Sauer & Walker, 1959; Sidman, Miale & Feder, 1959; Fujita, 1963), it follows that each nucleus is obliged to wait its turn for a vacancy at the surface of the canal. Therefore, the minimum time one of the daughter nuclei of a mitosis would take to return to the surface of the central canal would be 15 times the duration of mitosis. By the time the column was ten nuclei deep the generation time would be 30 times the mitotic duration. Thus, if nuclear migration to the cell apex continues, the minimum permissible generation time will increase with increasing pseudostratification unless there is an associated reduction in the duration of mitosis. This is borne out by the work of Jelínek (1959), Källén (1961) and Fujita (1962) on chick embryos, which indicated that the generation time of ependymal cells increased with the degree of development of the ependymal layer. All these authors also detected an associated increase in the mitotic duration, a curiously counter-productive happening in this situation. Jelínek (1959), for example, obtained an estimate of 39 minutes for the mitotic duration in ependymal cells in the spinal cord segment of the neural tube at 3 days of incubation, increasing to 149 minutes at 6 days. No such data is available for the mouse, although Atlas & Bond (1965) have calculated a time of about 1 hour for the mitotic duration in an unidentified segment of the neural tube of an 11-day mouse embryo, which, from their illustrations, appeared to be about 4–5 loosely packed nuclei in depth. Their estimate of 11 hours for the generative cycle is of the same order of magnitude as that obtained by multiplying the mitotic duration by the number of overlying nuclei (about 10 in this case, allowing for the loose packing).

It therefore seems that the pseudostratified structure of the ependymal layer makes it a proliferative trap, and that one or other or all of the difficulties which have been outlined above will operate to limit, or slow down, the precursor cell accumulation required under the first rule. The second rule of neurogenesis could be formulated thus: *given the pseudostratified structure of the ependymal layer, accumulation of neuron precursors will be limited by cell population congestion unless there is a corresponding increase in the surface area of the central canal, or a modification of the pseudostratified structure allowing mitotic dispersal.* Although dispersal of mitotic figures occurs fairly early in the development of such initially columnar epithelia as that of the oesophagus (Smart, 1970) and olfactory area of the nose (Smart, 1971*b*), the neural epithelium appears to be curiously reluctant to follow suit.

In the present study the relation between mitotic activity, degree of pseudostratification, and extent of differentiation was investigated, and a careful search was made for non-surface mitotic figures in the ependymal layer. The spinal cord segment of the neural tube was used because it gives rise to the least complicated division of the central nervous system and has a simple proliferative pattern. The results suggest that even in the generation of the relatively small neuronal population of this area the proposed rules can be seen to operate.

MATERIAL AND METHODS

The material consisted of mouse embryos of 10, 11, 12, 13, 14 and 15 days of post-conceptional age. These ages span the period of nerve cell production in the major part of the spinal cord. The embryos were fixed in Carnoy's solution, embedded in paraffin wax, serially sectioned at 6 μm in the transverse plane and stained with haematoxylin and eosin. All embryos were killed between 10 a.m. and noon. At least two embryos were available for each age group. The following procedures were carried out:

(1) A suitable cross-section of the developing spinal cord was put under a $\times 50$ oil immersion lens of a Leitz SM-Lux binocular microscope with $\times 10$ eyepieces, one of which contained an ocular micrometer. The position of the section was then adjusted so that the scale of the micrometer lay alongside the central canal surface of the ependymal layer. The number of mitotic figures lying at the surface of the canal adjacent to each division of the micrometer scale was then recorded. This provided an index of the number of mitotic figures per unit length of the canal surface and will be referred to subsequently as the *surface index*. The unit used was the arbitrary one of one division of the ocular micrometer. On calibration this was found to be 62 μm. It is therefore possible to compare, if necessary, the counts presented here with counts done with different lens systems by converting both to some common unit such as one of a 100 μm. This could be done to the results in this paper by multiplying the figures for each division by $\frac{100}{62}$.

(2) The same procedure was then repeated on the same section, this time recording the number of mitotic figures occurring in the ependymal layer away from the surface of the central canal at the level of each division of the micrometer. Care was taken to exclude the fairly numerous mitotic figures occurring in the nucleated blood cells which were present in the capillaries at this stage of development.

Counts 1 and 2 were made on 20 alternate serial sections at various levels of the developing cord at each age. Alternate sections were used in order to avoid counting the same mitotic figure twice, as might happen in adjacent sections. The *surface index* for each division of the micrometer scale was calculated by taking the average number of mitotic figures per division for the 20 sections counted. In sections in which the counts ended on an incomplete division the last count was calculated proportionately, either alone or summed into the last complete division.

(3) A typical section from the middle of a series on which the foregoing counts had been made was then projected with a projection microscope on to graph paper at a standard magnification, and the outlines of the neural tube, central canal, ependymal, and mantle layers were drawn in. For convenience, the magnification was arranged so that each division of the micrometer corresponded to 2 cm on the graph paper. The outline of the ependymal layer was then turned into its own histogram by dividing it into columns based on the micrometer divisions as in Figs. 2–11. The area of each column was then calculated by counting squares on the graph paper. By dividing the area so obtained into the sum of the average number of the mitotic figures occurring at the surface of the central canal and within the layer, an index of the number of mitotic figures per unit area was obtained for each division of the histogram. This will be referred to subsequently as the *area index*. The area of each column

is related to the number of nuclei present, so this index is a measure of the fraction of mitotic nuclei in the total population of ependymal cells in each column. Its main inaccuracies are that the periphery of the ependymal layer is not always easy to define exactly, and it takes no account of any possible changes in nuclear packing density, or of possible changes in the space occupied by blood vessels. The more desirable *mitotic index* is virtually impossible to calculate in the ependymal layer, as extreme nuclear crowding makes it difficult to distinguish the boundaries of all interphase nuclei and they therefore cannot be counted.

In all, some 30 counts were made at different levels of the developing spinal cord at different ages. They were then assembled into two series. The first series comprised counts made on sections at the level of the forelimb bud at each age period. Although during growth the spinal cord, vertebral column and forelimb bud do not maintain the same level relative to each other, there was little variation in the degree of maturation for quite a large segment of the upper end of the developing cord at a particular age, and the sections at the level of the forelimb bud at each age were considered to be comparable. The counts were made on two embryos at each age period. Both gave similar results, but due to different degrees of obliquity of cut giving different lengths to the surface of the central canal, the counts could not be pooled. In view of the similarity of the results, the counts from only one animal at each age period are presented in this report.

A second series comprised counts made on sections showing different degrees of maturation from different levels of the developing spinal cord without strict reference to the age of the embryo from which they came. These were arranged into order according to their state of maturity. As the results of the counts corresponded closely to the pattern of maturation only a selection is presented – those from different areas of maturation in an embryo of 13 days of post-conceptional age.

(4) The location, stage of mitosis, and orientation of the cleavage plane of dividing cells occurring within the ependymal layer, but excluding those at the surface of the central canal, were also recorded by counting these figures in 50 alternate serial sections of the neural tube at the level of the forelimb bud at each age period. Once again care was taken to exclude endothelial cell mitosis or figures occurring in nucleated blood cells. The location of a figure was recorded by counting the number of ependymal nuclei which intervened between it and the surface of the central canal; the layer of nuclei at the surface of the canal was designated layer 0, the next layer 1 and so on. Counts 4 and 5 were made under a $\times 100$ oil immersion lens and revealed many more mitotic figures, particularly early prophases (Fig. 12), than were apparent using the $\times 50$ lens. This was distressing from the point of view of calculating the area index but comment on its significance is made in the Discussion.

(5) The orientation of mitotic figures at the central canal surface was next determined by counting the orientation of a minimum of a total of 100 metaphases, anaphases and telophases at each age group at the level of the forelimb bud. The orientations were put into vertical, oblique and horizontal categories according to the angle made by the plane of cleavage with the surface of the central canal (Smart, 1970). Records for the dorsal and ventral halves were kept separately to see if mitotic orientation played any part in the dorso-ventral differentiation process.

RESULTS

Surface index

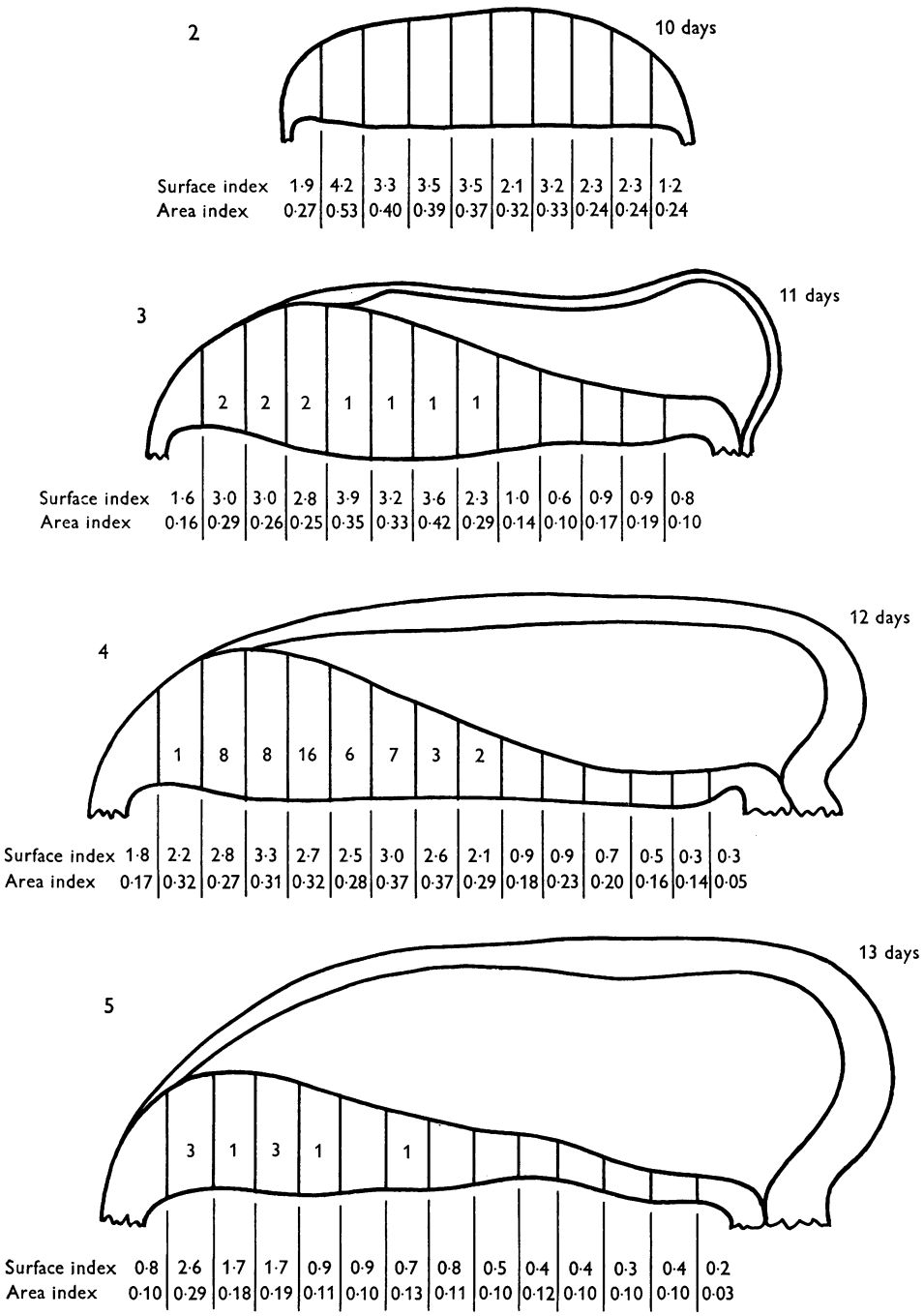
The changes in the surface index (average number of mitotic figures per unit length of central canal) are set out in Figs. 2–11 in the top line of figures written under the columns of the ependymal layer to which they correspond. The average number of figures in each developmental stage is less at the dorsal extremity of the alar lamina, but rises ventrally to decline once more towards the ventral end of the basal plate. In the series taken at the level of the forelimb bud, the index in the dorsal half of the sections (alar lamina) is about the same at 10 and 11 days of post-conceptional age (Figs. 2, 3), shows a slight decline at 12 days (Fig. 4), which progresses through the 13-day stages (Figs. 5, 6), until at 14 days (Fig. 7) the index becomes low throughout. In the ventral half of the sections (basal plate) the fall in the surface index is steep between 10 and 11 days, and thereafter remains at a roughly uniform low level, although at 14 days it shows a small increase in all three specimens of this age which were examined.

The relation of the surface index to differentiation is not clear-cut. There is no immediate decline in the surface index as it is traced ventrally under the advancing front of differentiating cells of the mantle layer (Figs. 3–5).

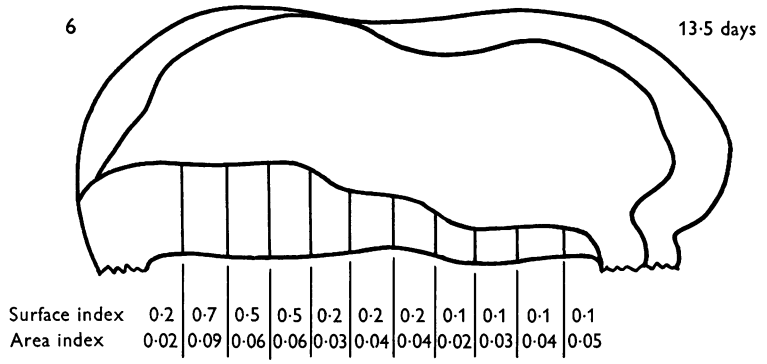
Area index

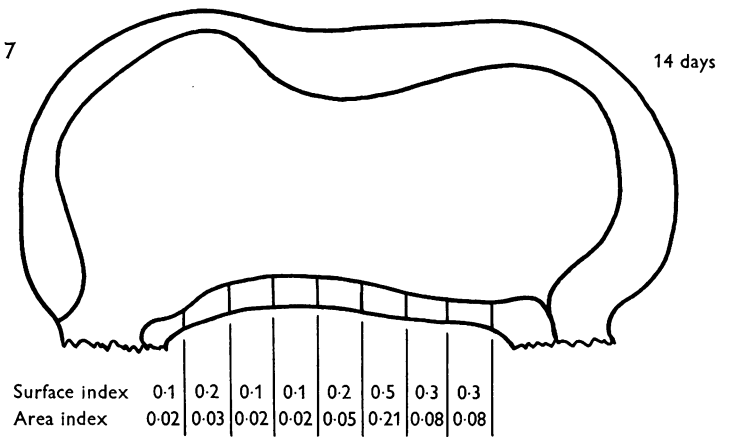
The changes in the area index, i.e. the average number of mitotic figures per unit area of the ependymal layer, corresponding to each column, are set out in the lower line of figures written under the individual columns in Figs. 2–11. There is a dorsoventral gradient which follows that of the surface index. There is, however, one important difference; mitotic activity does not decline at the same rate as the cell population of the ependymal layer. Thus, although the surface index may remain constant under the areas of differentiation, it is related to a smaller population of ependymal cells, and this produces a lesser fall in the area index. Thus at 11 and 12 days (Figs. 3, 4) the area of maximum mitotic activity in the ependymal layer appears to be under the advancing front of the mantle layer, where a decrease in ependymal layer population is beginning (Fig. 3 columns 5–7; Fig. 4 columns 7, 8). A similar effect is found in the more ventral regions of the basal plate. For example, in the 12 day specimen (Fig. 4) readings of 0·9, 0·9, 0·7 for the surface index in the ventral half are about one-third to one-quarter of the maximum value of 3·3 occurring in the dorsal half. The corresponding values for the area index are 0·18, 0·23, 0·20, which are about one-half to two-thirds of its value of 0·32, at the column where the ependymal layer has its maximum thickness.

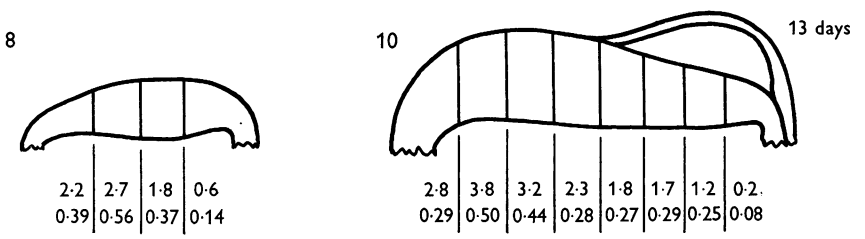
A similar pattern of variation of the surface and area indices can be observed in the second series of sections composed of different developmental stages from the same age period (Figs. 8–11). Here the difference between the surface and area indices is more striking. The values for the former are similar to those found more rostrally, but as they are related to relatively small ependymal cell populations the corresponding area indices are higher than are obtained from the more highly pseudostratified state of the rostral parts of the layer.

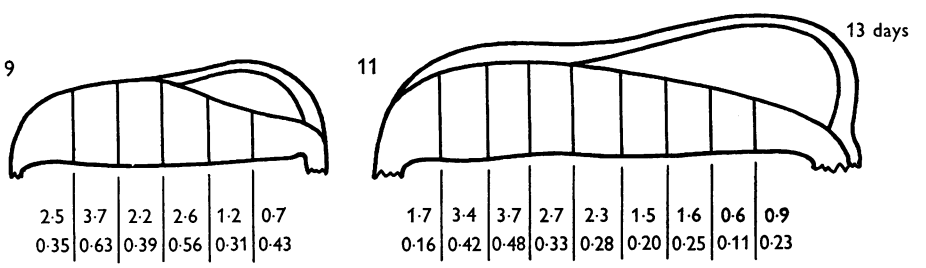


Figs. 2–5. Outlines of transverse hemisections of neural tube of mouse embryos of 10, 11, 12 and 13 days of post-conceptional age at the level of forelimb bud. The lumen of the tube lies inferiorly, and its dorsal and ventral aspects lie at the left and right hand sides of the page respectively. The outlines of ependymal, mantle and marginal layers are shown. The ependymal layer has been divided into columns each the width of one division of the scale of the ocular micrometer used in the counts (63 μm). The upper line of figures under each outline represents the surface index or the average number of mitotic figures found at the surface of the central canal at the base of each column. The lower line of figures is the area index or average number of mitotic figures per unit area of each column. The figures inscribed within the columns represent the total number of mitotic figures detected lying away from the central canal surface. All counts were made on the same 20 alternate serial sections at each age.









Figs. 6–11. Further outlines of hemisections of neural tube as in Figs. 2–5. Fig. 6: neural tube at 13½ days post-conceptional age. Fig. 7: neural tube at 14 days post-conceptional age. Note large area occupied by posterior horn. Figs. 8–11: neural tube at various levels caudal to the hind limb bud in an embryo of 13 days post-conceptional age.

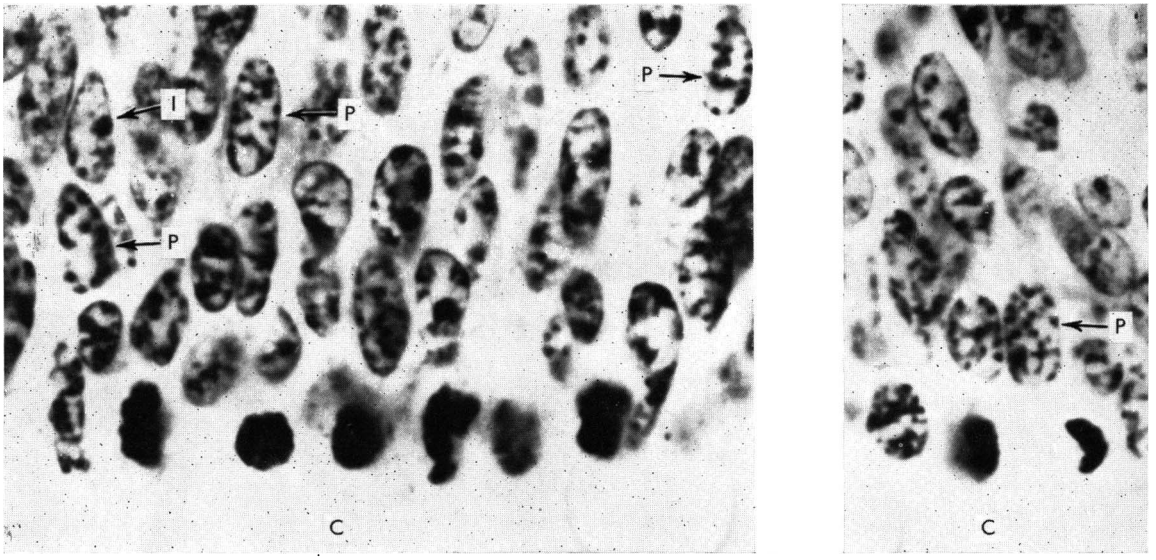


Fig. 12. Photomicrographs of 4 μm thick sections of ependymal layer of mouse embryo of 12 days post-conceptional age stained with haematoxylin and eosin. The area shown is the inner part of the layer adjacent to the surface of the central canal C. Note barrier of mitotic figures at the surface of canal, and early prophases P in non-surface nuclear layers. A typical interphase nucleus I is marked for comparison. *ca* $\times 1000$.

Location of mitotic figures

The distribution of mitotic figures occurring in the ependymal layer away from the surface of the central canal is given in Table 1. Such figures were found at all stages between 10 and 13 days of post-conceptional age but were most frequent at 12 days. At each age period they were most numerous in layer 1. However, any stage of mitosis was liable to be encountered in any layer in the 11, 12 and 13 day animals, and these figures appeared to be fairly evenly distributed at all levels.

Orientation of mitotic figures

At all ages 90–95 % of figures at the central canal surface were vertically orientated to give a plane of cleavage at right angles to the central canal. At all stages there was a small percentage which appeared to cleave in a plane parallel to the canal surface as if to form a superficial and deep daughter cell. The incidence of such cleavages did not seem to have any relation to differentiation, for they were as common in the dorsal as the ventral half of the tube, and in equivalent areas before and after the onset of differentiation. Figures occurring within the layer showed less regularity of cleavage plane and at all age periods showed a tendency to random orientation. For example, at 12 days, when they were most numerous, of a total of 117 figures whose orientation could be determined 40 % were vertical, 15 % oblique and 45 % horizontal.

DISCUSSION

The results demonstrate a dorso-ventral gradient of mitotic activity in the ependymal layer at all levels of the spinal cord segment of the neural tube that were examined (Figs. 2–11). This gradient can be divided into three stages, as in the diagrams in Figs. 14 and 15. In stage 1 proliferation occurs without differentiation and the precursor population grows. In stage 2 differentiation commences but is balanced by proliferation and the precursor pool consequently remains undiminished. In stage 3 differentiation exceeds proliferation and the precursor pool shrinks. It is convenient to subdivide this last stage into stage 3*a*, in which the area index is as great as the index for stage 1, and stage 3*b*, in which it is less than the stage 1 index. The area index, which is a rough measure of mitotic activity, is of the same order of magnitude in stages 1, 2 and 3*a*, and the growth pattern would therefore appear to be as follows.

As ependymal proliferation increases the area of the central canal, the dorsal lip moves further dorsally (as in Fig. 13), followed by the advancing front of cells which are differentiating into the mantle layer. The most dorsal extension of this advancing front marks an area where incipient differentiation is occurring at the same rate as precursors are being produced (stage 2). More ventrally the rate of differentiation increases (stage 3*a*) and finally slows down towards the floor plate (stage 3*b*). The consistently low area index at the extreme dorsal part of the ependymal layer may indicate that this forms a reservoir of cells ready to enter stage 1 when required.

The diagram in Fig. 15 can thus be read downwards in two ways: either as a profile of the ependymal layer from roof to floor plate during any stage of its proliferative life, or as the sequence each area of the ependymal layer follows during neuron formation. The latter use may require amplification. For example, the ventral end of the ependymal layer goes rapidly through the sequence, tarrying longest in stage 3*b*; the middle segment tarries longest in stage 2, and the dorsal segment in stage 1, thereafter moving rapidly through stages 2 and 3. Anything that delays the progression through this sequence while maintaining an undiminished area index increases cell production.

The results obtained are therefore consistent with the first rule suggested in the introduction. The dorsal part of the ependymal layer, which gives rise to the more populous posterior horn, delays differentiation until the end of the neuron-producing period and then undergoes quick and complete differentiation, the ependymal layer all but vanishing at 14 days post-conception (Fig. 7). Conversely, the fewer motor cells of the anterior horn are associated with early differentiation and a low area index. Inspection of Fig. 7 shows that the posterior horn occupies a greater area than the anterior. The cell density of the posterior horn is also greater by a factor of approximately 1·6:1 at this stage, as judged by counting the average number of nuclei appearing in ten oil immersion fields in the posterior and anterior horns.

There is also clear evidence that mitotic dispersal is taking place in the dorsal part of the ependymal layer, where pseudostratification is most pronounced. Table 1 reveals that from 10 days post-conception onwards there is an increasing number of prophases, particularly early prophases (Fig. 12), and metaphases lying immediately outside the layer of nuclei at the surface of the canal. This suggests that nuclei are entering visible mitosis before a vacancy is available for them at the surface of the canal. In addition to this surface population a second, smaller population of

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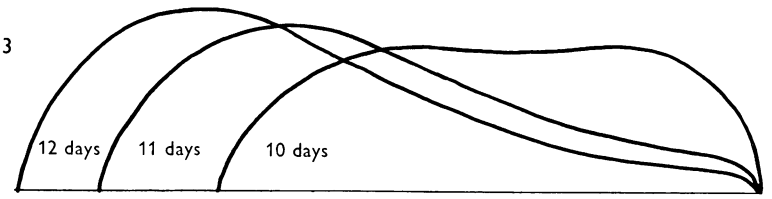

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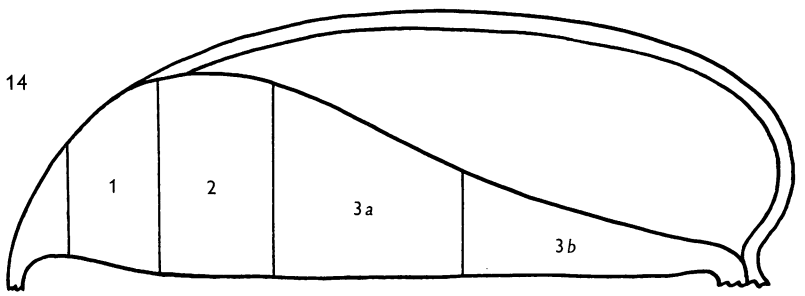

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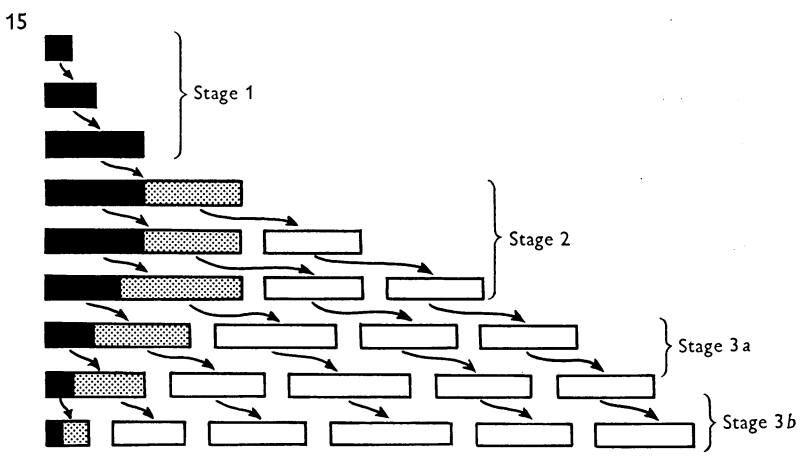

figures at all stages of mitosis can be distinguished, occurring at any level of the ependymal layer, indicating the presence of a group of cells in which the mitotic and migratory cycles are completely out of phase, or which have abandoned migrating altogether. The planes of cleavage of these non-surface dividing cells tend to be randomly orientated, indicating that the factors imposing the orderly vertical orientation characteristic of the surface figures are no longer operating. Both populations are greatest at 12 days, just prior to the decline of the ependymal layer which commences at 13 days. Thus at 12 days the choking of the ependymal layer surface

seems to be complete, and adumbrates the metaphase accumulations at the canal surface obtained by Watterson (1965) after the administration of colchicine. The area index of the dorsal half of the ependymal layer is actually higher at 12 days than indicated in Fig. 4, if the early prophase group of nuclei are included. The index counts were done under a × 50 objective, whereas the location counts were performed under the more revealing × 100 objective, which allowed the identification of many more early prophase nuclei of the type illustrated in Fig. 12, as well as other figures hidden among the densely packed nuclei in other layers. The presence of mitotic figures outside the surface layer of nuclei, in relation to the areas of maximum pseudostratification, does seem to suggest the operation of the second rule proposed in the introduction: that is, in areas where ependymal proliferation is not matched by a corresponding increase in the area of the central canal, continued proliferation leads to increasing pseudostratification and eventually to dispersal of mitotic figures.

The tenacity with which ependymal nuclei continue to migrate to the apical surface of the epithelium is interesting when it is remembered that mitotic dispersal occurs relatively early in the histogenesis of the structurally somewhat similar olfactory epithelium (Smart, 1971 *b*).

The second series taken through different levels of the 13 day embryo spinal cord showing different degrees of maturation presents a similar pattern (Figs. 8–11). These sections came from areas caudal to the hind limb bud, where the eventual cell population is smaller than in areas associated with innervation of the limbs. The ependymal layer does not pseudostratify to the extent observed at the anterior limb bud level (compare the depths of the ependymal layer in Figs. 3 and 11), reflecting the lesser need for the accumulation of precursors. The lesser degree of pseudostratification is also associated with little sign of mitotic congestion and enables a high mitotic index to be obtained from a modest number of apical figures (compare the surface and area indices in Figs. 8–11). This underlines the fact that the pseudostratified

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Fig. 13. Superimposed outlines of lateral margins of ependymal layer drawn from cross-sections of the neural tube at 10, 11 and 12 days of post-conceptional age at the level of the forelimb bud.

Fig. 14. Diagram of cross-section of spinal cord segment of early neural tube with the ependymal layer marked off into different proliferative stages.

Fig. 15. Diagram intended to depict the type of proliferation present in the different parts of the ependymal layer as designated in Fig. 14. The undifferentiated proliferative compartment is represented in black, and the stippled part of each box is the component about to differentiate out along the direction of the arrow into the mantle zone. The unshaded boxes represent the successive differentiated non-proliferative populations which have been added to the mantle layer. Stage 1 represents proliferation without differentiation, the undifferentiated compartment consequently increasing in size. Stage 2 represents a balance between proliferation and differentiation. The proliferative compartment doubles in size at each generation, but loses half its population by differentiation. Stage 3*a* represents a state where differentiation exceeds proliferation although the rate of proliferation remains the same: that is, the reduced number of cells remaining in the proliferative compartment doubles at each generation. Stage 3*b* represents a state where differentiation exceeds proliferation but the rate of differentiation has decreased. This is depicted by the final proliferative compartment being less than twice the size of the penultimate solid black compartment.

The stages in Figs. 14 and 15 can thus be matched against each other. The diagram in Fig. 15 can also be used to depict the proliferative sequence gone through by each area of the neural tube, different areas spending different lengths of time in each stage.

Table 1. *Distribution of non-surface mitotic figures occurring in thee pendymal layer of the upper thoracic segments of the developing neural tube of mouse embryos between* 10 *and* 13 *days post-conception*

(The counts represent the total number of such mitotic figures in 50 alternate serial sections at each age. The figures in column 1 indicate the number of nuclei intervening between the figure and the surface of the central canal.)

Age, post-conception	Layer	Early prophase	Prophase	Metaphase	Anaphase	Telophase
10 days	1	47	12	2	—	1
	2	1	1	—	—	1
	3	2	2	2	—	—
	4	1	1	—	2	—
	5	—	—	1	—	—
	6	—	—	1	—	—
	7	—	—	—	—	—
	8	—	—	—	—	—
	9	—	—	—	—	—
	10	—	—	—	—	—
11 days	1	90	13	3	—	—
	2	8	1	1	—	—
	3	—	—	2	—	1
	4	—	—	2	—	1
	5	1	2	—	—	—
	6	—	1	—	—	—
	7	—	—	1	—	1
	8	—	1	1	—	1
	9	—	1	2	—	—
	10	—	—	1	—	—
12 days	1	147	12	13	—	3
	2	11	6	8	1	1
	3	4	8	8	2	8
	4	1	5	7	3	4
	5	1	3	8	4	4
	6	—	2	8	1	7
	7	—	3	10	1	3
	8	—	3	9	1	3
	9	—	4	3	2	4
	10	—	2	10	2	7
13 days	1	24	4	4	—	—
	2	7	—	2	3	1
	3	2	1	5	—	—
	4	—	—	1	—	—
	5	—	—	—	1	—
	6	—	—	1	—	1
	7	—	—	2	—	1
	8	—	—	1	—	1
	9	—	1	1	—	—
	10	—	2	1	—	—

structure of the ependymal layer is quite efficient at producing cells in small numbers but is ill-adapted for quantity production unless it is free to increase in area.

Certain other features of the results are worthy of comment although they are not directly concerned with the main argument. The differences between the linear and area indices are interesting. Both show a dorso-ventral decline but the area index less

so; that is, although the number of figures on the surface of the canal is decreasing the population of cells giving rise to them is decreasing even faster. The proliferative activity of the decreased ventrally located population of ependymal cells is therefore not as low as the impression given by the relatively few mitotic figures observed. Thus, although the surface index is the most accurate to calculate, it is not of much value unless referred to the size of the cell population from which it is derived. The increase in surface and area indices at the ventral end of the ependymal layer at 14 days post-conception, when the layer is otherwise in the process of rapid decline, is small, but consistent, being observed in all three specimens of 14 day embryos examined. As by 15 days the layer is further reduced in area and mitotic activity, this final small increase in cell production is interesting because it does not fit an otherwise tidy pattern.

The incidence of horizontal cleavages is at all stages of development too small (about 5 %) to account for the release of a sufficient number of cells from the ependymal layer to form the cell population of the mantle layer from the more peripherally located daughter cell of such cleavages. It follows that release of differentiating cells from the ependymal layers must occur either by the cell loosening itself from the terminal bar network at the epithelial surface or by some modification or suppression of the events which are associated with formation of the terminal bars at the end of mitosis. The decrease in area of the central canal from 13 days onward also seems to result from continued loss of cells from the ependymal layer and not from fusion of opposing sides, as occurs in parts of the ventricular system of the brain. The contraction of the lumen is compensated for by thickening of the roof and floor plate by elongation of their component cells, as was demonstrated in Cajal's classic preparations. In none of the sections of the developing spinal cord was anything resembling a sulcus limitans observed, nor was there any observable morphological division between sensory and motor areas in the early stages of development. The impression was gained, however, that the posterior horn originated mainly from the final massive differentiation of the dorsal extremity of the ependymal layer between 13 and 14 days post-conception (Figs. 5–7) and not from any strictly morphologically delineated segment of the tube.

SUMMARY

Cell production by the ependymal layer of the spinal cord segment of the neural tube of mouse embryos of 10–15 days of post-conceptional age was studied. The number of mitotic figures per unit length of the central canal surface and per unit area of the ependymal layer was recorded. A particular search was made in the ependymal layer for mitotic figures which were located away from the surface of the central canal. The location and stage of mitosis of these figures were recorded.

In a series of sections taken at the level of the forelimb bud it was found that neuron formation in the ventral half of the neural tube commenced about 11 days of post-conceptional age and continued at a decreasing rate until about 14 days. The neurons of the posterior horn, on the other hand, formed at about 13 days of post-conceptional age from the rapid differentiation of a large population of more rapidly dividing ependymal cells. Non-surface mitotic figures were most common in the dorsal half of the tube at 12 days, just prior to differentiation. Prophases and meta-

phases located in the nuclear layer adjacent to the layer of nuclei at the surface of the canal were the most common.

These results are taken as supporting the thesis which initiated the investigation, namely that cell production by the neural tube is governed by two main factors. The first is that more nerve cells can be produced in a given number of generations if differentiation is delayed, and that this leads to the accumulation of undifferentiated ependymal cells. The second is that accumulation of ependymal cells in the pseudostratified ependymal layer will be limited by cell population congestion unless there is a corresponding increase in area of the central canal or a modification of the strictly pseudostratified structure of the layer allowing mitotic dispersal.

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